Program/Abstract # 213
Drosophila apontic acts as a feedback inhibitor of JAK/STAT signaling and is required to limit an invasive cell population
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Gradients of signaling are used repeatedly in development to instruct cells to acquire different properties or behaviors dependent on the level of signaling. In the epithelium of the *Drosophila* ovary, high levels of JAK/STAT signaling specify a small number of cells to become the border cells, which must undergo a stereotyped migration. Cells with lower levels of STAT activation remain within the epithelial layer. How this gradient of signaling is translated into a binary decision to migrate or not was unclear. We found that the *apontic* (*apt*) gene is required for this process. In *apt* mutants, the migratory population was expanded and cells were unable to detach efficiently from the epithelium. This phenotype resembled gain-of-function of JAK/STAT activity. Gain of function of APT also mimicked loss of function of STAT and its key downstream target, SLBO. APT expression was induced by STAT, which was further supported by DNA binding assays. Together, this suggests that APT functions as a feedback inhibitor of the STAT pathway. We conclude that a regulatory circuit between STAT, APT, and SLBO functions to convert an initially graded signal into an all-or-nothing activation of JAK/STAT and thus into proper cell specification and migration. A mathematical model incorporating the relationships of these proteins can accurately simulate wild-type and mutant phenotypes, supporting the idea that our three component system is sufficient to translate a gradient into discrete cell identities.

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Program/Abstract # 214
Sequential actions of Pax3 and Pax7 drive xanthophore development in zebrafish neural crest
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The *Pax3/7* gene family has a fundamental and conserved role during neural crest formation. In people, *PAX3* mutation causes Waardenburg syndrome, and murine Pax3 is essential for pigment formation. However, it is unclear exactly how Pax3 functions within the neural crest. Here we show that *pax3* is expressed before other *pax3/7* members, including duplicated *pax3b*, *pax7* and *pax7b* genes, early in zebrafish neural crest development. Knockdown of Pax3 protein by antisense morpholino oligonucleotides results in defective fate specification of xanthophores, with complete ablation in the trunk. Other pigment lineages are specified and differentiate. As a consequence of xanthophore loss, expression of *pax7*, a marker of the xanthophore lineage, is reduced in the neural crest. Morpholino knockdown of Pax7 protein shows that Pax7 itself is dispensable for xanthophore fate specification, although yellow pigmentation is reduced. Loss of xanthophores after reduction of Pax3 correlates with a delay in melanoblast differentiation followed by significant increase in melanophores, suggestive of a Pax3-driven fate switch within a chromatophore precursor or stem cell. Analysis of other neural crest derivatives reveals that, in the absence of Pax3, the enteric nervous system is ablated from its inception. Therefore, Pax3 in zebrafish is required for specification of two specific lineages of neural crest, xanthophores and enteric neurons.

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Program/Abstract # 215
The zebrafish mutants lpy and myx exhibit loss of skeletogenic cranial neural crest
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The vertebrate cranial neural crest (CNC) gives rise to both non-ectomesenchymal derivatives (e.g. neurons, glia, and pigment cells) and ectomesenchymal derivatives (e.g. cartilage and bone). How the ectomesenchyme lineage of the CNC is specified is not well understood. In order to understand ectomesenchyme specification, we identified two zebrafish mutants, lpy and myx, which display specific loss of the ectomesenchyme-derived head skeleton. The expression of early neural crest markers (including sox9b, sox10, snai2, and foxd3) is reduced or completely absent in lpy embryos at early neurulation, suggesting a defect and consequent delay in neural crest specification. However, we have demonstrated that other CNC derivatives appear to be unaffected in lpy mutants, with the notable exception of melanophore pigment cells which are variably lost. Furthermore, transplantation of wild-type CNC precursors into lpy embryos shows that the *lpy* gene is required autonomously within the CNC for normal crest induction and development of the head skeleton. Thus, lpy and myx represent two potential key players in the specification and/or development of the cranial neural crest and the ectomesenchyme lineage. We will present analyses of CNC cell fate in wild-type and lpy mutants, together with more preliminary studies of the recessive myx mutant. In addition, we have mapped the semi-dominant lpy mutation to a small interval on linkage group 3 and will present progress towards identifying the *lpy* gene.

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Program/Abstract # 216
Notch resolves mixed neural identities in the zebrafish epiphysis
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Manipulation of Notch activity alters neuronal subtype identity in vertebrate neuronal lineages. Nonetheless, it remains controversial as to whether Notch activity diversifies cell fate by regulating the timing of neurogenesis or acts directly in neuronal subtype specification. Here, we address the role of Notch in the zebrafish epiphysis, a simple structure containing only two neural subtypes, projection neurons and photoreceptors. Reducing the activity of the Notch pathway results in an excess of projection neurons at the expense of photoreceptors as well as an increase in cells retaining a mixed identity. However, while forced activation of the pathway inhibits the projection neuron fate, it does not promote photoreceptor identity. As birthdating experiments show that projection neurons and photoreceptors are born simultaneously, Notch acts directly during neuronal specification rather than by controlling the timing of neurogenesis. Finally, our data suggests that two distinct signals are required for photoreceptor fate specification: one for the induction of the photoreceptor fate and the other, involving Notch, for the inhibition of projection neuron traits. We propose a novel model in

which Notch resolves mixed neural identities by repressing an undesired genetic program.

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Program/Abstract # 217
Notch signaling has differing effects on subpopulations of retinal progenitor cells in zebrafish retinal development
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Cell-to-cell interactions mediated by the *Notch* pathway play critical roles in regulating the temporal pattern of neurogenesis. In the vertebrate retina, upon binding a ligand encoded by the *DSL* (*Delta/Serrate/lag-2*) genes, Notch signaling suppresses neuronal differentiation and promotes continued proliferation. Inhibition of Notch signaling results in an increase in the number of neuroblasts that differentiate as ganglion cells and cone photoreceptors, two early cell fates. Conversely, overexpression of a constitutively active form of Notch (NICD) promotes Muller glial differentiation, a later cell fate. Here we tested for a coordinated role of Notch in zebrafish retinal progenitor cell proliferation and subsequent differentiation using the *mindbomb* allele (*mib*^{ta52b}), which lacks Notch function, and two heat-shock transgenic lines that allow for temporal regulation of Notch signaling. In *mib* mutant embryos, BrdU and PH3 labeling revealed that in the absence of Notch signaling, a subset of retinal progenitor cells exits the cell cycle early and differentiates as ganglion cells, while the remainder of progenitor cells continues to proliferate in a spatial and temporal pattern similar to the wild-type pattern. Temporal expression of the NICD resulted in increased Muller glia differentiation at all time points tested as has been previously demonstrated, though mitotic cell numbers were not in excess of their wild-type siblings. Taken together, these data suggest that Notch signaling has differing effects on subpopulations of retinal progenitor cells in zebrafish retinal development.

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Program/Abstract # 218
Lots-of-rods (*lor*) regulates photoreceptor subtype specification in zebrafish
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The number and distribution of neurons generated during development of the vertebrate retina are tightly regulated and critical for image formation. This arrangement is particularly apparent in the highly ordered, crystalline-like mosaic of the photoreceptors in the teleost. Using as a model the mosaic pattern of photoreceptors in the zebrafish, we have undertaken a genetic screen to identify loci that are essential for photoreceptor subtype specification. We identified the locus, *lot-of-rods* (*lor*), that when mutated results in an increased number of rods and a reduced number of ultraviolet-sensitive (UV) cones in larvae and adults. This phenotype is the opposite of that observed in enhanced S-cone syndrome and the rd7 mouse. Quantitative and spatial pattern analyses suggest an approximate one-to-one exchange of rods for UV cones in the mutant compared to wild-type larvae with little alterations in red, green or blue cones. Linkage analysis and complementation testing indicate that the *lor* locus encodes a T-box transcription factor. In genetic chimeras, *lor* mutant cells failed to generate UV cones in a wild-type host. Conversely, wild-type cells displayed the capacity to differentiate into UV cones when transplanted into a mutant host. The identification of a novel function for a T-box gene in photoreceptor development provides a much needed system to understand the molecular network regulating neuronal subtype specification in the retina and dissect the UV vision pathway in a vertebrate. Supported by R1EY017753.

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Program/Abstract # 219
Intra-endodermal interactions are required for pancreatic β-cell induction
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The cellular origin of signals that regulate pancreatic β-cell induction is not clearly defined. Here, we investigate the seeming paradox that Hedgehog/Smoothened signaling functions during gastrulation to promote pancreatic β-cell development in zebrafish, yet has an inhibitory role during later stages of pancreas development in amniotes. Our cell transplantation experiments reveal that in zebrafish, Smoothened function is not required in β-cell precursors. At early somitogenesis stages, when the zebrafish endoderm first forms a sheet, pancreatic β-cell precursors lie closest to the midline; however, the requirement for Smoothened lies in their lateral neighbors, which ultimately give rise to the exocrine pancreas and intestine. Thus, pancreatic β-cell induction requires Smoothened function cell non-autonomously during gastrulation, to allow subsequent intra-endodermal interactions. These results clarify the function of Hedgehog signaling in pancreas development, identify an unexpected cellular source of factors that regulate β-cell specification, and uncover complex patterning and signaling interactions within the endoderm.

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Program/Abstract # 220
PAR-1 phosphorylates the ubiquitin ligase Mind bomb to repress Notch signaling and promote vertebrate neurogenesis
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Epithelial cell polarity is dynamically controlled during early development and is often misregulated in cancer. The serine/threonine kinases PAR-1 and atypical protein kinase C (aPKC) are important players in the establishment of epithelial polarity. Our previous study demonstrated that PAR-1 functions downstream of aPKC to stimulate ciliated cell differentiation in *Xenopus* ectoderm via a Notch signaling-dependent mechanism. Here we show that the same signaling cassette is used during neuronal differentiation of mammalian neural progenitors in vitro. We demonstrate that a crucial molecular substrate for PAR-1 is Mind bomb (MIB), a ubiquitin ligase that promotes Notch signaling by modulating Delta ligand trafficking and activity. The phosphorylation of MIB by PAR-1 results in MIB degradation, repression of Delta-Notch signaling and stimulation of neuronal differentiation. Our data suggest that PAR-1 acts in ectodermal cell fate determination by modulating Notch signaling